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(54) Title: USE OF SIMMONDSINE

(57) Abstract

The food intake by animals, including humans, can be influenced by administering a suitable dose of simmondsine. The appetite is hereby inhibited and weight loss effected. The simmondsine can be administered as such or in the form of de-oiled jojoba flour. Derivatives and/or analogues of simmondsine can further be applied such as simmondsine-2'-ferulate or aglucons of simmondsine. A suitable method for isolating and purifying simmondsine (and/or analogues thereof) comprises: extracting de-oiled jojoba flour (preferably with acetone), fractionating the extract by means of adsorption chromatography, filtering the fractions containing the simmondsine, fractionating the filtrate again by means of adsorption chromatography and crystallizing the simmondsine from the obtained fractions. Practical applications of the invention relate particularly to reduction of the food intake in mother animals of livestock for fattening in addition to stimulating recuperation growth in animals for slaughter, in diets for pets etc.

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USE OF SIMMONDSINE

The present invention relates to the use of simmondsine for influencing the food intake by animals.

In particular cases it may be important to inhibit the growth of animals or humans. With animals this is particularly the case in the animal breeding industry, such as the chick-fattening sector, wherein mother animals, in contrast to the animals for slaughter themselves, may not gain rapidly in weight. Due to genetic selection animals intended for slaughter, and therefore also the future mother animals, have a strong urge to eat a great deal. Owing to this characteristic the chickens are quickly ready for consumption.

A rapid weight gain due to high food intake by future mother animals can however result, among other things, in reduced fertility, defects of the legs and an increased mortality rate. In order to prevent these drawbacks and moreover increase the egg production and sitting ability and to reduce chick mortality the mother animals are placed on a strict feed limitation diet. This means for instance that the farmer must weigh the animals regularly and administer feed in accordance with the weight development and sometimes deprive the animals of food for one or two successive days. This is not only very labour-intensive for the farmer but also very unpleasant for the animals in question. Such a method therefore also encounters growing opposition from animal protection groups.

With respect to the carcass quality (the relationship between meat and fat) in animals for slaughter, it can even be advantageaous to fast the animals for a short time during growth. After this period, when the animals again have unlimited food at their disposal, there occurs a so-called recuperation growth, also called the catch-up growth. The food conversion and carcass quality are hereby positively influenced. During fasting of animals, the same

problems as hereabove are demonstrated for the mother animals.

House pets such as dogs, cats, guinea pigs, doves and suchlike can become overweight through too much feeding. Such an overweight condition is obviously not beneficial to the health of the animal. However subjecting these animals to fasting is not easy because the animal is used to a certain amount of food a day. It is unpleasant for the animal if it can not receive its usual amount of food and this can lead to troublesome behaviour in the animals as a consequence. Thus, an animal friendly manner of food reduction is desired.

Such as appears from the above, there are a variety of conceivable situations wherein a regulated reduction in the food intake by animals is desired, without being unpleasant or having disadvantageous side effects for the animal.

It has been found that the substance simmondsine and its derivatives and analogues, whether or not in an isolated or pure form, has an effect on the food intake of animals, so that without any unpleasantness on the part of the animal, the desired food reduction can be achieved by the animals' owner or the farmer.

The present invention therefore relates to the use of simmondsine and/or its derivatives and/or its analogues in a suitable dose for influencing the food intake by animals such as fish, birds and mammals.

By the term "simmondsine" is understood, in the accompanying claims, all sources and administrable forms of simmondsine and substances derived from or related to simmondsine which display biological activity consistant with pure simmondsine. This not only includes, for instance, simmondsine and its derivatives and analogues in pure or unpure form, but also includes jojoba flour which can be, if necessary, heated and/or de-oiled. Jojoba flour can be used separately or as part of an admixture. Combinations of simmondsine and jojoba flour are also possible.

By the term "pure simmondsine(s)" is meant, in the description, the isolated more or less pure forms of simmondsine and its derivatives. The terms "pure simmondsine" and "pure simmondsines" can be used together. When the term "jojoba flour" is used, all the herewith, whether or not, treated, for instance de-oiled, heated etc. forms of the flour are meant which can be obtained from the jojoba plant.

Up until now the opinion was that simmondsine was a poisonous substance and that the presence thereof in vegetable food such as seeds or flour was undesireable. The American patent 4,148,928 describes for instance a method for removing these undesireable elements from jojoba flour.

In a publication from Verbiscar in the Journal of Agricultural Food Chemistry 26(6): 1456-1459 (1978) it is suggested that laboratory mice, rats and poultry do not thrive on jojoba flour and that this is caused by the presence of certain toxic glycosides including simmondsine. As a result of this, the flour was thought not to be suitable for an animal food. Ngou Ngoupayou has stated in Poultry Science 61: 1692-1696 (1982) that simmondsine and simmondsine-2'-ferulate are poisonous for rodents and poultry.

It has now been found however that the working of simmondsine is based on increasing the feeling of satisfaction by releasing the hormone cholecystokinine into the intestines, and not through the toxicity of the constituents. As a result of this increased feeling of satisfaction, a strong decrease in the food intake comes about which leads to weight loss as a consequence.

Apart from this, it appears by both young animals as well as young people (adolescents) that as a result of simmondsine a higher maintenance metabolism also results whereby weight loss occurs faster than by normal fasting. The above mentioned hormone cholecystokinine stimulates the enzyme production by the pancreas. This increased enzyme production leads to a protein loss, especially of essential amino acids. As a reaction to this the body of the growing

animals and humans increases the production of tri-iodothyronine (T3), a thyroid hormone, which stimulates energy burning in the tissues. Accordingly an extra fast weight loss occurs.

One application of the use of simmondsine is, for instance, found in the keeping of stock animals for fattening such as chick-mother animals. It has been found from extensive tests that admixing to the feed of only 4% simmondsine, containing jojoba flour de-oiled with hexane, already causes a growth curve corresponding with what is desired by the animal farmer. It can generally be said that the mixing of 1-10% jojoba flour, or an amount of simmondsine active substances which corresponds with the simmondsine activity of 0.075-0.50% pure simmondsine, added to the feed as of life week 3 until and including life week 23, reduces food intake of the chick-mother animals to such an extent that the desired body weight is achieved in the weeks 20-23. At the moment in many of the genetic lines used as chick-mother animals this weight is ± 2.3 to ± 2.5 kg. Naturally by changing the genetic lines, the amount of simmondsine to be added can be adjusted to agree perfectly with the needs of the line.

Another application of simmondsine, according to the present invention, has been found in weight loss diets of small pets, such as dogs, cats, guinea pigs, rabbits, hamsters, rats, mice, chinchilla's, doves, canaries, parrots etc. Pure simmondsine can in such instances be mixed in an amount of 0.05%-1.0% with the feed which itself can have a reduced energy content, and which can be in a dry or wet form, pasteurized, extruded or sterilized. Simmondsine can also be dissolved in drinking water or can be applied in the form of tablets, capsules or other forms of administering medicine. When de-oiled and/or heated integral jojoba flour is used, the dosing is dependant on the simmondsine content (simmondsine and its analogues simmondsine-2'-ferulate, but also other simmondsine-like substances), this content being between 0.5 and 20%. This jojoba flour can be administered in the same way as pure simmondsine but not as a solution.

Dependant on the dosing a reduction in food intake from 5-50%, but mostly between 5-25%, can be achieved. The treatment can, if desired, be carried out over a shorter or longer time period, for instance between 1 day and 1 year.

The recuperation growth in animals for slaughter, such as poultry for slaughter, for instance, can be achieved by a 10-20% food reduction, introduced during the second, third and possible fourth week whereafter the animals are allowed to eat ad libitum. A food reduction of 10-20% can be brought about by mixing 0.1-0.25% simmondsine activity or 2-5% jojoba flour into the feed. 0.1-0.25% simmondsine activity agrees with 0.1-0.25% simmondsine or 0.15-0.37% simmondsine-2'-ferulate. The simmondsine activity of simmondsine-2'-ferulate is 0.68 x the activity of simmondsine itself. The recuperation growth can apart from poultry also be induced in pigs or cattle for fattening.

In sheep and cattle breeding fat formation in the animals can be countered by limiting the food intake by means of from 2-10% jojoba flour .

Simmondsine also has the same effect in humans and can thus also be used for curbing the appetite of people who must, or want to, follow a diet for example.

The use of simmondsine according to the invention is therefore suitable for limiting the food intake in all sorts of animals including humans, but finds practical application particularly in livestock for fattening and livestock mother animals, for countering too strong growth and for bringing about weight loss for pets.

Simmondsine can be administered to animals in diverse ways, for example in the form of pure simmondsine or as an admixture of de-oiled or possibly heated jojoba flour to the feed. In addition, an extra simmondsine extract from the jojoba plant can also be added to the de-oiled jojoba flour.

The jojoba flour can be separated from its oil in various different ways. Preferably the flour is de-oiled by means of hexane.

De-oiled jojoba flour for administering to animals is preferably heated for a while in order to break up the trypsine inhibitors present in the jojoba flour. Heating can be carried out for 30-120 minutes at 100-120°C for example. When heated jojoba flour is used, it appears that an enduring, and very well predictable dose-dependant food intake reduction can be achieved.

Apart from simmondsine itself, derivatives and/or analogues of simmondsine can also be used such as simmondsine-2'-ferulate or aglucones of simmondsine.

The invention further relates to a method for isolating and purifying simmondsine and/or derivatives and/or analogues thereof, comprising the steps of:

a) extracting de-oiled jojoba flour in order to obtain an extract;

b) separating the extract into fractions by means of adsorption chromatography;

c) filtering the fractions containing the simmondsine and/or derivatives and/or analogues thereof to obtain a filtrate;

d) separating the filtrate into fractions again by means of adsorption chromatography; and

e) crystallizing the simmondsine and/or derivatives and/or analogues thereof.

It is likewise possible to concentrate the extract respectively the filtrate prior to the chromatography step.

In a preferred method according to the invention the extraction is performed with acetone, the first chromatography takes place on a silica gel column with acetone as eluant, the filtration takes place over activated carbon and the second chromatography takes place on a silica gel column with chloroform/methanol (95:5) as eluant. Concentration preferably takes place by means of dissolving the extract respectively the filtrate in methanol and subsequently evaporating the methanol under vacuum.

The present invention will be further elucidated with reference to the examples below, which are not however intended to limit the invention in any way.

EXAMPLE 1

Influence of simmondsine in feed on the thyroid hormone concentrations in adult rats.

In the research that has led to the present invention, it has been found that simmondsine indirectly influences the manufacture of the thyroid hormones in the body. In growing animals tha manufacture is stimulated, while in adult animals it is inhibited. In this experiment the influence of simmondsine in a pure form and in the form of de-oiled jojoba flour on the production of T3, T4 and TSH hormones, is investigated in adult Wistar rats.

1. The influence of pure simmondsine

1.1 Test conditions.

The influence of 0.5% simmondsine in feed on the thyroid hormone concentrations is determined in 30 adult male Wistar rats of ± 350 g which are divided into 3 groups of 10 animals. Group C consists of control animals. The animals of group S receive for 11 days 0.5% HPLC pure simmondsine mixed into their feed, group PF receives precisely the same amount of ordinary feed as the S group consumes (so-called "pair-fed" group).

Blood sampling takes place by cutting off a portion of the tail. The blood is collected in heparinized vessels and the plasma is immediately centrifuged off and stored in the deep-freeze until hormone assays are possible. After 2, 6, 10, 14, 18 and 22 hours blood is taken in order to determine the circadian rhythm of the hormone concentrations.

Hormone assays are performed by means of RIA tests on the thyroid hormones T3 (tri-iodothyronine), T4 and TSH. The 2 x 10 controls are pooled into one group.

1.2. Results

The animals of group S exhibit a feed intake inhibition of about 50%. Table 1 shows the plasma concentration (ng/ml) of T3 in male Wistar rats after 11 days administration of 0.5% pure simmondsine in the feed.

The different indices (a, b, c) designate statistically significant differences ($p<0.05$). The lower values represent the S.E.M..

Table 1

Plasma concentrations of T3 at 0.5% Simmondsine

	2	6	10	14	18	22 u
C	0.657a 0.020	0.780a 0.042	0.978a 0.045	0.739a 0.033	0.536a 0.019	0.529a 0.034
S	0.367b 0.027	0.286b 0.013	0.338b 0.039	0.388b 0.046	0.290b 0.028	0.444b 0.028
PF	0.460c 0.020	0.444c 0.027	0.419b 0.044	0.481b 0.030	0.380c 0.025	0.490b 0.013

In table 2 are shown the plasma concentrations (ng/ml) of T4 in male Wistar rats after 11 days admixture of 0.5% simmondsine in the feed. The different indices (a, b, c) designate statistically significant differences ($p<0.05$). The lower values represent the S.E.M..

Table 2

Plasma concentration of T4 at 0.5% simmondsine

	2	6	10	14	18	22 u
C	64.50a 5.30	54.02a 5.25	53.38a 4.24	55.38a 5.15	49.99a 3.16	53.02a 4.60
S	28.56b 2.53	23.07b 1.94	23.02b 1.68	32.44b 4.70	36.28b 6.26	31.39b 1.41
PF	41.47c 4.54	38.59c 3.40	33.34c 2.50	39.91c 4.27	46.77a 5.46	42.97c 4.27

Shown in table 3 are the plasma TSH-concentrations (ng/ml) in male Wistar rats after 11 days admixture of 0.5% simmondsine in the feed. The different indices (a, b, c) designate statistically significant differences ($p<0.05$). The lower values represent the S.E.M..

Table 3

Plasma concentrations of TSH at 0.5% Simmondsine

	2	6	10	14	18	22 u
C	1.616a 0.158	1.741a 0.301	2.499a 0.364	2.745a 0.321	1.906a 0.182	1.269a 0.101
S	0.981b 0.088	1.361b 0.541	1.599b 0.289	1.543b 0.088	1.056b 0.151	0.213a 0.143
PF	0.932b 0.081	1.274b 0.110	1.506b 0.237	1.395b 0.185	0.923b 0.066	1.082a 0.391

1.3 Discussion

It can be seen from the above tables that there is a fall in TSH, T3 and T4 compared to the controls, which can be expected because the animals are fasting. The T3 and T4 fall is however more strongly pronounced (although not always statistically significant) in the animals treated with simmondsine. This can be explained by a slight protein deficiency. The TSH fall can be wholly explained by the fasting. The treatment with simmondsine does not have any supplementary effect.

2. The influence of 10% jojoba flour de-oiled with hexane in feed

2.1. Test conditions

30 adult male Wistar rats of ± 350 g are divided into groups of 10 animals. Group C consists of control animals, the animals of group JJ receive for 11 days 10% de-oiled jojoba flour mixed through their feed, the animals of group PF receive precisely the same amount of ordinary feed as the JJ group consumes (pair-fed group).

Blood sampling and hormone assays are performed in the same manner as under 1.1 above.

2.2. Results

In the case of the JJ group the feed intake inhibition amounts to about 50%. The weight loss is the same as with

animals receiving 0.5% simmondsine. Table 4 shows the plasma T3 concentration (ng/ml) in male Wistar rats after 11 days admixing of 10% de-oiled jojoba flour. The different indices (a, b, c) designate statistically significant differences ($p<0.05$). The lower values represent the S.E.M..

Table 4

Plasma concentration of T3 at 10% de-oiled jojoba flour

	2	6	10	14	18	22 u
C	0.657a 0.020	0.780a 0.042	0.678a 0.045	0.739a 0.033	0.536a 0.019	0.529a 0.034
JJ	0.471b 0.026	0.445b 0.031	0.547b 0.049	0.599b 0.052	0.415b 0.024	0.504b 0.028
PF	0.519b 0.034	0.622c 0.035	0.581a,b 0.035	0.668a,b 0.046	0.491c 0.024	0.556a 0.020

Shown in table 5 is the plasma T4 concentration (ng/ml) in male Wistar rats after 11 days admixing of 10% de-oiled jojoba flour. The different indices (a, b, c) designate statistically significant differences ($p<0.05$). The lower values represent the S.E.M..

Table 5

Plasma concentration of T4 at 10% de-oiled jojoba flour

	2	6	10	14	18	22 u
C	64.50a 5.30	54.02a 5.25	53.38a 4.24	55.84a 5.15	49.99a 3.16	53.02a 4.60
JJ	47.75b 4.20	44.75b 2.91	55.42a 5.19	59.25a,b 6.17	51.72a 2.62	48.60a,b 4.10
PF	47.20b 7.77	55.27a 3.16	55.96a 6.37	63.38b 5.88	49.51a 6.12	54.36a 4.39

Table 6 shows the plasma THS concentrations (ng/ml) in male Wistar rats after 11 days admixing of 10%

de-oiled jojoba flour. The different indices designate statistically significant differences ($p<0.05$). The lower values represent the S.E.M..

Table 6

Plasma concentration of TSH at 10% de-oiled jojoba flour

	2	6	10	14	18	22 u
C	1.161a 0.158	1.741a 0.301	2.499a 0.364	2.745a 0.321	1.906a 0.182	1.269a 0.101
JJ	1.304b 0.111	1.496b 0.158	2.014b 0.174	1.759b 0.154	1.129b 0.107	1.204a 0.087
PF	1.271b 0.067	1.407b 0.107	1.847b 0.146	1.720b 0.116	1.192b 0.091	1.224a 0.097

2.3 Discussion

There is a fall in TSH, T3 and T4 concentration relative to the controls, which can be expected because the animals are fasting. The T3 and T4 fall is more strongly pronounced in the group treated with jojoba flour, although this is not everywhere statistically significant. The fall in TSH content can be wholly explained by the fasting. The treatment with jojoba flour has no influence hereon.

3. Conclusion

In adult rats the administering of 0.5% pure simmondsine or 10% jojoba flour de-oiled with hexane results in a feed intake reduction, weight loss and a fall in the T3 and T4 content relative to the controls. The thyroid hormone fall is usually more strongly pronounced in the treated animals than in the pair-fed rats. The fall relative to the controls can be explained by the lower feed intake. The greater fall in the case of the treated animals could be explained by a relative protein deficiency, which in adult animals normally causes a slight fall in T3 and T4 (de Mello, M.A.R. et al., Circulating thyroid hormone levels in

young pregnant rats and their fetuses: effect of malnutrition. Ann. Nutr. Metab., 1989, 33, 181-187).

The TSH content falls under the influence of fasting but no influence as such can be seen from the treatment with simmondsine or jojoba flour.

EXAMPLE 2

Comparison of the effect of 0.15% simmondsine and 3% de-oiled jojoba flour on the growth, feed intake and food conversion in growing female rats.

1. Introduction

0.15% simmondsine, which is mixed into feed, is compared with 3% jojoba flour de-oiled with hexane and containing 3.6% simmondsine and 0.8% simmondsine-2'-ferulate (which gives 0.108% simmondsine and 0.024% ferulate in the flour). From other tests is known that simmondsine-2'-ferulate causes approximately 68% of the feed intake inhibition of simmondsine (research of the inventors). This therefore means that 3% jojoba flour contains the equivalent of 0.12% active simmondsine. In preparatory tests it was found that the feed intake inhibition amounts in both situations to about 20%, despite the difference in active simmondsines. Other elements must consequently be present in the flour which play a minor part in the feed intake inhibition. Known are the trypsin inhibitors of protein type, complex tannins, bitter flavourings and the like. These tests seek to verify the effect of both admixtures on the growth performance.

2. Test conditions

50 just weaned female Wistar rats divided into 5 groups of 10 animals are followed for 8 weeks. The feed intake and the weight increase are measured daily. Then calculated weekly herefrom are the relative growth (weight week 2 - weight week 1/weight week 1 * 100) and the food conversion (grams feed required per gram weight increase). Group C consists of controls, group S consists of animals treated with 0.15% simmondsine in feed, group JJ contains

animals treated with 3% jojoba flour de-oiled with hexane in the feed, group PFS consists of rats which are pair-fed with S and group PFJ contains the rats which are pair-fed with JJ. PFS and PFJ tests start one week later on animals born one week later, so that at the start they are the same age and weigh as much as C, S and JJ.

3. Results

The results are shown in the following tables. Table 7 shows the weight evolution in grams (only average weights). The different indices (a, b, c) designate statistically significant differences.

Table 7

Weight Evolution

	start	week 4	week 8
C	91.1a	171.3a	203.6a
S	93.6a	138.2b	161.8b
JJ	93.9a	138.1b	164.7b
PFS	93.4a	149.5c	178.5c
PFJ	93.0a	147.9c	176.7c

Table 8 shows the feed intake (gram/week/animal) (only mean values).

Table 8

Feed Intake

	1	2	3	4	5	6	7	8
C	92	102	102	107	108	112	107	102
S	75	84	83	84	86	84	81	84
JJ	75	85	80	84	83	84	81	81
PFS	75	84	83	84	86	84	81	84
PFJ	75	85	80	84	83	84	81	81

Table 9 shows the relative growth. The different indices (a, b, c) designate statistically significant differences.

Table 9

Relative growth

	1	2	3	4	5	6	7	8	aver. 1-4	aver. 5-6
C	21.5	12.8	14.0	9.7	6.3	2.6	3.3	2.4	14.5a	3.65a
S	16.1	7.2	8.7	4.7	5.5	3.0	3.5	1.7	9.2b	3.4a
JJ	14.1	7.0	6.9	5.5	5.5	2.5	3.8	2.5	8.4b	3.7a
PFS	14.3	13.9	9.4	6.5	5.6	4.0	3.0	3.3	11.0c	3.9a
PFJ	15.5	12.5	9.0	4.7	4.7	4.9	2.3	2.8	10.4c	3.7a

Table 10 shows the food conversion. The different indices (a, b, c) designate statistically significant differences.

Table 10

Food Conversion

	1	2	3	4	5	6	7	8	aver. 1-4	aver. 5-6
C	3.7	4.7	4.7	6.4	9.3	23.3	16.7	18.3	4.9a	16.9a
S	4.3	9.6	7.3	12.2	10.7	18.8	15.0	25.1	8.4b	17.4a
JJ	4.7	10.1	8.8	11.1	9.5	22.7	13.7	16.5	8.7b	15.6a
PFS	5.1	4.7	6.3	8.7	9.7	12.6	15.9	15.6	6.2c	13.5b
PFJ	4.5	5.5	6.6	11.6	11.6	10.8	21.6	12.9	7.0c	14.3b

The effect of 0.15% simmondsine and 3% jojoba flour is the same for feed intake inhibition, growth, relative growth and feed conversion. Feed intake amounts to ± 80% of that of the control group C. Relative growth and food conversion in group S and group JJ are less than in group C in the first half of the test but the same as the controls in the second

half. The relative growth and the food conversion of the pair-feds is better in the first half than in the groups S and JJ but becomes the same in the second half. The relative growth and food conversion in the case of the pair-feds is less during the first four weeks than in the controls but the relative growth becomes equal to and the food conversion even becomes better than in the controls at the end of the test.

We know from previous work that at the beginning of these tests no T3 fall can be seen in group JJ (so that the T3 concentration remains at the level of the controls), while there is however a fall in group PFJ. At the end of the test the T3 concentration in group JJ falls again to the level of the pair-feds (Cokelaere et al., J. Agric. Food Chem., 1993, 41, 1444-1448). In the case of growing animals with feed limitation a food conversion is always observed that is less than in normally fed animals. This can be explained in that the energy and the substances used for growth are in fact only those which remain after the requirements of the maintenance metabolism have been satisfied. This excess is always smaller in the case of animals with feed limitation than in the controls. In the case of feed-limitation however, the maintenance metabolism falls among other things due to a fall in the T3 concentration.

The poor food conversion and poorer relative growth in group JJ can therefore be partly explained by the fasting itself. The poorer results in the first half of the test, compared to the pair-feds, can be explained by the higher T3 values in group JJ. At the end this T3 content coincides with that of the pair-feds, at the moment the animals are growing just as rapidly with the same food conversion as the pair-feds. Since we see the same results in group S as in group JJ and we known from preliminary tests (not published) that a T3 rise can also be discerned in young animals which are given simmondsine, we may assume that the explanation in the case of group JJ also applies in the case of group S.

4. Conclusion

It can be concluded that despite the differences in simmondsine-active equivalents in group S and group JJ, the results for food intake and growth performance are the same.

EXAMPLE 3

Influence on the pancreas weight of 0.15% simmondsine and 3% jojoba flour de-oiled with hexane

Simmondsine increases the cholecystokinine concentration in the body. Cholecystokinine in turn stimulates enzyme production of the pancreas. These experiments have been carried out in order to assertain whether the weight of the pancreas changes under influence of simmondsine.

1. Test conditions

50 adult female Wistar rats of approximately 220 grams are coupled (checked by copulation test) with normal Wistar male rats. The animals are divided into 5 x 10 rats.

During 16 days of gestation the animals are fed in five different ways. Group C consisting of controls are fed normally, group S receives feed with 0.15% simmondsine, group JJ receives feed with 3% jojoba flour de-oiled with hexane, group PFS is pair-fed daily with the S group and group PFJ is pair-fed daily with the JJ group.

Shown here are only the body weight (BW), weight of the pancreas and weight of the pancreas expressed as % of BW.

2. Results

Table 11 shows the mean body weight, pancreas weight and pancreas weight as % of the body weight. Each group numbers 10 animals. The different indices (a, b, c) designate statistically significant differences ($p<0.05$).

Table 11

	weight (g)	pancreas weight (g)	pancreas weight in % of BW
C	252a	0.795a	0.315a
S	229b	0.900b	0.393b
JJ	220b	0.948b	0.341b
PFS	235b	0.684c	0.299a
PFJ	212b	0.680c	0.321a

3. Discussion

A marked enlargement of the pancreas can be seen in the groups S and JJ relative to the groups PFS and PFJ, but there is also a slight increase relative to the controls.

EXAMPLE 4

Influence of pure simmondsine on the food intake in chickens and effect of the administering method

Chickens are an important target group for the application of simmondsine according to the invention. In this experiment, the influence of pure simmondsine on chickens is examined.

1. Test conditions

20 spring chicks of about 400 grams receive feed ad libitum. They are divided into 5 groups of 4 animals and their feed intake is checked after 1, 2, 4, 6, 8 and 24 hours.

Group C consists of control animals, group IT receives 250 mg/kg simmondsine intubated into the stomach, group IV receives 250 mg/kg administered intravenously, group IP receives 250 mg/kg administered intraperitoneally, group IVO receives 140 mg/animal calculated per day mixed into the feed.

2. **Results**

Table 12 shows the average food intake expressed per kg body weight in growing chickens for slaughter.

Table 12

	1	2	4	6	8	24
C	30.49a	11.63b	26.36a	24.80b	22.97b	92.51a
IT	20.98b	8.05b	17.97b	21.26b	16.28b	83.99b
IP	27.86a	7.72b	17.42b	35.40c	16.17b	87.33a
IV	18.13b	11.08b	16.95b	27.51c	24.28a	70.61d
IVO	23.95b	13.14b	17.99b	18.13b	25.96a	73.02d

3. **Discussion**

The simmondsine exhibits a marked effect on the food intake in IT, IV and IVO. In IT it acts certainly for 8 hours, in IV a maximum of 4 hours, in IVO permanently. When the controls eat less (due to sensation of satisfaction) a less pronounced additional influence of simmondsine is observable, which points once again to a satisfaction-increasing effect of simmondsine.

EXAMPLE 5

Influence of jojoba flour on the growth, food consumption and plasma hormone concentrations of chicks.

1. **Determining of jojoba flour dosage**

1.1. **Test conditions**

80 three week-old chicks were weighed individually and divided into 8 groups of 10 chicks each. The mean initial body weight of these groups was the same. In one of the groups (R) the food intake was limited in accordance with the recommendations of the breeder (weeks 4 and 5: 45 g per chick per day; weeks 6 and 7: 47 g per chick per day).. The other groups had unlimited access to commercially available feed supplemented with 0 (control), 2, 4, 6, 8, 10

or 12% jojoba flour de-oiled with hexane (0-12% JO). The de-oiled jojoba flour contained 4.15% simmondsine and 0.7% simmondsine-2'-ferulate. Each week up to week 7 all chicks were individually weighed and the weekly food intake per group recorded. At weeks 4 and 7 a blood sample was collected by a puncture in the wing artery of all chicks. Blood was taken from the R chicks after their daily feed ration was made available to them. Blood samples were collected in heparinized tubes, centrifuged and the plasma stored at -20°C until the determining of the hormone content.

1.2. Results

The effects of jojoba flour supplement on body weight and on weekly food intake are shown in fig. 1A and 1B. In this graph are shown the mean values for 10 chicks for 0% JO (□), 2% JO (O), 4% JO (O), 6% JO (▲), 8% JO (▼), 10% JO (△), 12% JO (▽), and feed limitation (■). A significant effect ($p < 0.0001$) of the diet treatment on the body weight was observed from week 4. The 0% JO chicks were significantly heavier than the 2% JO chicks, followed by the 4% JO and R chicks. No significant differences could be observed between the body weights of the 6, 8, 10, and 12% JO chicks.

Between weeks 3 and 7 the R and 4% JO chicks consumed respectively 1.288 and 2.862 g per animal, which was 35 and 78% of the total food intake of the 0% JO chicks. The addition of 6% or more jojoba flour to the feed pushed the food intake down further. At least 12% jojoba flour was necessary to cause a fall in the food intake to the level of the R chicks. Between weeks 3 and 7 the 2, 4, 6, 8, 10 and 12% JO chicks consumed respectively 64.3, 114.5, 121.6, 145.3, 141.0 and 145.1 g JO flour per chick.

The growth hormone, insulin growth factor-I, T4 and T3 levels in plasma at weeks 4 and 7 are shown in table 13,

Table 13

	Week 4					Week 7			
Treatment	GH	IGF-I	T_3	T_4		GH	IGF-I	T_3	T_4
					ng/ml				
0% JO	32.4^{c}	24.4^{a}	2.22^{a}	5.7^{d}		16.1^{d}	29.9^{a}	1.39^{b}	8.2^{b}
2% JO	60.3^{bc}	18.3^{b}	2.01^{ab}	8.7^{c}		25.6^{d}	24.9^{b}	2.13^{a}	12.1^{a}
4% JO	92.9^{ab}	16.1^{bc}	2.08^{ab}	8.6^{c}		31.1^{cd}	21.5^{b}	1.80^{ab}	8.6^{ab}
6% JO	124.0^{a}	14.0^{c}	1.82^{bc}	8.6^{c}		76.8^{ab}	16.2^{c}	1.71^{ab}	7.4^{b}
8% JO	83.7^{abc}	10.0^{d}	1.47^{cd}	10.8^{ab}		61.9^{bc}	16.6^{c}	1.76^{ab}	7.1^{b}
10% JO	85.8^{abc}	10.5^{d}	1.31^{d}	9.1^{bc}		86.5^{ab}	11.0^{cd}	1.30^{b}	8.7^{ab}
12% JO	123.6^{a}	10.6^{d}	1.44^{cd}	8.2^{c}		105.7^{a}	13.3^{d}	1.47^{b}	6.3^{b}
Restricted	79.4^{abc}	6.4^{e}	$.92^{e}$	12.1^{a}		29.1^{cd}	16.3^{c}	1.48^{b}	9.1^{ab}
SEM	6.46	.333	.045	.212		3.97	.555	.065	.418

$^{a-e}$Averages in a column without common superscript differ significantly (P<.05)

[1]Each value is the average of 10 values

At week 4 and compared to 0% JO values the mean plasma growth hormone concentrations increased after supplementation with jojoba flour, although a significant effect was only observed for the 4, 6 and 12% JO chicks. The plasma IGF-I concentrations of JO flour chicks were significantly lower compared to those of the 0% JO chicks and reached a minimum level at 8% JO supplementation. Compared to the 0% JO value, supplementation of 6% JO or more caused the plasma T3 level to fall sharply, while circulating plasma T4 concentrations were already significantly increased when 2% JO was added to the feed. The mean plasma growth hormone concentrations of R chicks did not vary from those of all other groups. The plasma IGF-I and T3 levels of the R chicks were however significantly lower than for all other groups, while the opposite was true for circulating plasma T4 concentrations.

The above experiments show that a decrease in the rate of growth was already achieved with 4% JO supplementation.

2. Comparison of JO chicks with a pair-fed group

2.1 Test conditions

40 three week-old chicks were weighed individually and divided equally into two groups which received the commercial feed with 0% or 4% jojoba flour. A week later 40 three week-old chicks were again weighed individually and divided into two groups. The food intake of 1 group was limited in accordance with the recommendations of the breeder. The other group (PF group) received on a daily basis the same quantity of the non-supplemented diet as that consumed by the 4% JO chicks. At intervals of a week up to 7 weeks all chicks were individually weighed and the food intake per group recorded. Blood sample were taken at week 4 and processed as described under 1.1 above.

2.2 Results

Despite the similar age, the average initial body weight at 3 weeks differed significantly between the two

series of 40 chicks each (fig. 2A). 0% JO = □, 4% JO = ●; pair-feeding = ○ and feed limitation = ■. The values are averages of 17 to 20 chicks per treatment. Despite the equal feed consumption of the 4% JO and the pair-fed (PF) chicks (fig. 2B), the 4% JO chicks gained less in body weight between 3 and 7 weeks than their PF opposite numbers (4% JO: 615 g/chick; PF: 796 g/chick). As already observed in the experiment described under 1.1, R and 4% JO chicks followed the same growth curve (fig. 2A).

Plasma growth hormone, insulin growth factor-I, T4 and T3 concentrations at week 4 are shown in table 14.

Table 14

Variable	0% JO	4% JO	PF	R
Plasma levels [1]				
GH, ng/mL	31.9 ± 4.7^{b}	51.7 ± 4.8^{a}	35.5 ± 2.2^{b}	57.4 ± 6.6^{a}
IGF-I, ng/mL	$25.0 \pm .8^{a}$	$14.2 \pm .6^{b}$	$11.1 \pm .8^{c}$	$6.2 \pm .3^{d}$
T_4, ng/mL	$11.4 \pm .8^{c}$	$12.2 \pm .7^{c}$	22.2 ± 1.8^{a}	16.7 ± 1.6^{b}
T_3, ng/mL	$1.43 \pm .07^{a}$	$1.33 \pm .09^{a}$	$.77 \pm .07^{b}$	$.61 \pm .05^{b}$
Feed passage time [2]				
min	208 ± 12^{a}	192 ± 15^{a}	177 ± 14^{ab}	147 ± 5^{b}
min/kg BW	199 ± 16^{b}	293 ± 31^{a}	203 ± 16^{b}	212 ± 13^{b}

^{a,b,c,d} averages within a row without common superscript differ significantly ($p<0.05$)
[1]values are averages ± SEM, n = 17 to 20 for each treatment.
[2]values are averages ± SEM, n = 8 for each treatment.

Jojoba flour supplement and food limitation increased the circulating plasma growth hormone compared to the 0% JO value. Pair-feeding had no significant effect on plasma growth hormone compared to 0% JO. Compared to 0% JO value

all diet treatments reduced the plasma IGF-I significantly. The lowest plasma IGF-I values were observed in R chicks. Plasma T3 quantities in 4% JO chicks were comparable to those of 0% JO chicks and significantly higher than those of the PF and R birds. The highest plasma T4 concentrations were observed in the PF chicks. Plasma T4 did not differ between 0% JO and 4% JO chicks, while plasma T4 of the R chicks lay between that of the 0% JO and the PF chicks.

EXAMPLE 6

Comparison of the effect of simmondsine and simmondsine-2'-ferulate on the food intake inhibition of rats

1. Introduction

Simmondsine and its analogues both show simmondsine activity. To discover whether the simmondsine activity of both substances correspond, the effect of both substances on the food intake of rats was studied.

2. Test conditions

The 24 hour food intake of 10 adult male rats (363 ± 6 grams) was measured during three days. After this, food was offered for three days to the rats, wherein 0.5 wt.% HPLC-pure simmondsine (S) was admixed. The 24 hours' food intake of the rats was measured daily. After a recovery period of 1 week, food was offered to the rats during three days wherein 0.5 weight per cent HPLC-pure simmondsine-2'-ferulate (SF) was admixed. Their daily food intake was again measured.

3. Results

The food intake in grams/animal/24 hours was 20.7 ± 0.43 for the control animals. Addition of 0.5% simmondsine gave a food intake of 7.57 ± 1.36 whilst 0.5% simmondsine-2'-ferulate resulted in a food intake of 12.47 ± 0.96.

4. Discussion

The above results yield a food intake decrease of 63% and 40% for S and SF respectively. SF therefore caused ± 63.5% of the food intake reduction of S. Since the molecular weight of S (375) is 68% that of SF (551), it can be said that S and SF cause the same food intake inhibition for the equimolecular amounts. When the concentration of SF (on a weight basis) in jojoba flour or animal feed is multiplied by a factor of 0.68, the resulting number corresponds with a certain concentration of pure simmondsine. From dose/response curves, it can therefore be deduced how much food intake inhibition will be brought about.

EXAMPLE 7

The effect of trypsine inhibitors and skin factors on the food intake inhibition caused by de-oiled jojoba flour.

1. Introduction

Tests (Cokelaere et al., J. Agric. Food Chem., 1992, 40, 1839-1842) have shown that pure simmondsine, extracted from the jojoba nut, reduces food intake. However decreased jojoba flour also contains trypsine inhibitors (Samac et al., Plant Physiol., 1981, 68,1339-1344; Sanchez-Luzero et al., Proc. Int. Conf. on Jojoba and its uses, 1983, 21-31), both in the protein containing parts as well as in the skin. The food intake inhibition which is caused by the addition of de-oiled jojoba flour can disadvantageously be influenced thereby.

The following tests have been carried out in order to show that trypsine inhibitors of proteine nature can be destroyed by heating and that the anti-trypsine activity of the skin does not play any significant role in the food intake reduction by de-oiled jojoba flour.

2. Test conditions

2.1 Determination of the skin content (on weight basis) in de-oiled jojoba flour.

1.3 kg of complete jojoba nuts (originating from Israel) were placed in boiling water for 30 minutes. After this the skin was removed by a cutting motion. After drying of the skin and evaporation of the moisture, the weight of the various elements was measured.

2.2 Dose response curves

To 4 groups of each 8 adult Wistar rats (300-350 grams) was added complete rat food in the form of flour, each for 7 days. Differing concentrations of the following substances were admixed in the feed.

(a) pure simmondsine,

(b) de-oiled integral jojoba flour,

(c) de-oiled integral and heated (1 hour at 120°C) jojoba flour, or

(d) de-oiled de-skinned and heated jojoba flour.

Between each 7 day treatment period was inserted a recuperation period of 7 days. The daily food intake was measured and compared with a 7 day control food intake for each group. The concentrations of pure simmondsine varied from 0.1% to 1%. The concentrations of jojoba flour varied from 2% to 20%.

The concentration of simmondsine (S) amounted to 4% in the de-skinned and heated flour and 4.8% in the integral flours; the concentration of simmondsine-2'-ferulate (SF) amounted to 1.4% in the three flours. The calculated concentrations of S and SF activity (= concentration of SF x 0.68; see Example 6 above) in the flours is shown in table 15. The integral jojoba flour originates from Israel and had been cold pressed (max. 60°C). It was de-oiled with hexane in a Soxhlet apparatus. Heated integral de-oiled flour was heated in a dry oven for 1 hour at 120°C. The de-skinned and heated flour was ground and de-oiled with hexane in a Soxhlet apparatus.

2.3 The effect on the relative growth and food conversion of de-skinned and heated jojoba flour, integral de-oiled jojoba flour, integral de-oiled and heated jojoba flour, pure skin factors and essential amino acids.

The de-skinned and heated flour, the integral flour, the heated integral flour and the skin factors were obtained as above. 11 Groups of each 8 just weaned male Wistar rats were given complete laboratory food for rats, in the form of flour, for 4 weeks. Apart from a control group (C), groups of 5 rats each were examined, that were given food according to the scheme below:

(a) flour with 5% de-skinned, heated and de-oiled jojoba flour (DS),

(b) 5% integral de-oiled jojoba flour (I),

(c) 0.25% pure simmondsine (SS),

(d) 5% de-oiled, de-skinned and heated jojoba flour supplemented with 0.5% of the three following essential amino acids: threonine, valine and methionine (DSAA),

(e) fine ground skin equivalent to 5% integral jojoba flour (5% x 23% on a weight basis) (SK).

The food intake was measured on a daily basis. A group of 8 rats was also pair-fed with these 5 groups (they received daily the same amount of food as their respective treated group had received the day before) (PDS, PI, PSS, PDSAA; PSK).

During week 3 and 4, the groups I and PI were separated into 2 groups. The first half followed the treatment of the first two weeks (f). THe second half received 5% heated de-oiled integral jojoba flour (IH) or were pair-fed with this (PIH) (g).

3. Results

3.1. Skin content (factor) in integral flour

The total weight of flour was 1,300 grams, of which 135 grams were skin parts. After boiling, 15 grams of crystalline material were obtained from the cooking fluid.

This means that the jojoba nut (originating from Israel) contained 11.5% skin. Converted, the integral jojoba flour contained 23% skin (factors) considering the jojoba nut consisted for ± 50% of liquid wax (oil).

3.2. The effect on the food intake inhibition: dose response

The results of the food intake inhibition tests are shown in table 15.

Table 15

Concentration of simmondsine active elements (pure simmondsine (S) + simmondsine-2'-ferulate x 0.68 (SF)) in rat feed.

Feed mixed with pure simmondsine	Feed mixed with de-skinned heated flour	Feed mixed with integral flour	Concentration of flour in the feed
0.1	0.099	0.115	2
0.15	0.149	0.173	3
0.25	0.248	0.288	5
0.50	0.495	0.575	10
0.75	0.743	0.863	15
1.00	0.99	1.142	20

The feed intake inhibition is illustrated in figure 3.

For like concentrations of simmondsine activity in the animal feed, adult male Wistar rats show a comparable food intake reduction, on the condition that the integral jojoba flour was sufficiently heated. The de-skinned jojoba flour was heated for 30 minutes during the de-skinning process and showed no supplementary food intake inhibition which can be associated with pure simmondsine. The non heated integral flour showed a much stronger food intake

inhibition, which can be removed by heating. The somewhat stronger food intake inhibition which was caused by heated integral flour with comparable concentrations of de-skinned flour can be explained by the somewhat higher concentrations of simmondsine active parts (see table 15).

3.4. The effect on the food intake, relative growth and food conversion

The results of the experiments relating to the food intake, relative growth and food conversion are given in table 16 to 18.

Table 16

Food intake as a % of the controls.

Week	1	2	3	4
DS	62.4	56.4	57.3	56.3
SS	71.2	62.2	62.3	61.4
DSAA	68.2	61.6	60.0	60.4
SK	86.3	97.0	98.2	95.2
I	45.9	40.0	38.2	37.1
IH	-	-	41.9	43.8

As can be expected from the the foregoing tests, 5% de-skinned, de-oiled and heated jojoba flour caused roughly 40% food intake inhibition, which is comparable with that caused by 0.25% pure simmondsine.

The relative growth and food conversion are disadvantageously influenced by the food intake inhibition. The presence of simmondsines in DS and SS strengthened this occurance especially in week 3, but weakened again in week 4. This reinforces what had previously been described for male and female growing rats (Cokelaere et al., J. Agric. Food Chem. 1993, 41, 1444-1448; Cokelaere et al., J. Agric. Food Chem., 1994, filed): the slow growth and worse food conversion observable in quickly growing animals is a

temporary occurrence as a result of the administering of pure simmondsine or simmondsine containing de-oiled, heated jojoba flour.

Table 17

Relative growth

Week	1	2	3	4	average 3/4
C	54.6	37.4	24.1	19.8	21.9
DS	21.4	18.3	14.2	12.2	13.2
PDS	22.1	19.2	19.6	12.7	16.2
SS	28.3	19.9	15.8	12.3	14.1
PSS	20.8	18.9	21.6	13.9	17.8
DSAA	23.5	19.7	15.7	14.0	14.9
PDSAA	20.5	19.1	13.0	16.4	14.7
SK	44.5	31.2	23.4	20.3	21.8
PSK	36.0	32.4	22.3	21.2	21.8
I	3.4	10.4	6.7	7.0	6.85
PI	5.6	13.8	11.3	8.4	9.8
IH			17.0	13.6	15.3
PIH			13.9	15.0	14.45

By supplementation with essential amino acids, (which are specifically lost by pancreas stimulation) the food conversion and the relative growth are normalized. This again reinforces the hypothesis that simmondsines cause food intake inhibition by releasing cholecystokinine, which in turn stimulates enzyme production in the pancreas. The latter causes a relative protein deficit which results in an increased thyroid hormone production and a higher metabolism.

Non heated integral flour causes a much stronger food intake inhibition and an even worse food conversion and relative growth. Switching over to heated integral flour causes a rapidly improved food conversion and relative growth, although after a two week pre-treatment with non-

heated integral jojoba flour, the food intake did not strongly improve.

The skin itself has little or no effect on the food intake, food conversion or growth rate.

Table 18

Food conversion

Week	1	2	3	4	average 3/4
C	2.3	2.98	3.52	3.6	3.56
DS	3.57	4.18	4.92	5.36	5.14
PDS	3.52	4.04	4.02	4.93	4.47
SS	3.14	4.08	4.60	5.89	4.89
PSS	3.93	4.30	3.39	4.31	3.85
DSAA	3.51	4.12	4.49	4.55	4.52
PDSAA	4.02	4.17	5.36	3.89	4.62
SK	2.34	3.48	3.83	3.61	3.72
PSK	2.69	3.32	3.72	3.42	3.57
I	16.7	6.18	8.39	7.59	7.99
PI	10.3	4.69	4.32	6.36	5.34
IH			3.78	4.42	4.10
PIH			4.27	3.81	4.04

4. Discussion

Heated and de-oiled integral jojoba flour causes an enduring, predictable dose-dependant food intake inhibition amongst rats, which can be completely explained by the presence of simmondsine and simmondsine-2'-ferulate. Trypsine inhibitors from the protein fraction and the skin can be destroyed by heating. If the integral jojoba flour is not heated, there occurs a very bad growth performance and food conversion. The de-oiled integral jojoba flour must be sufficienly heated, for use with animals, in order to destroy the trypsine inhibitors.

EXAMPLE 8

Isolation and purification of simmondsine

This example illustrates the method for isolating and purifying simmondsine from jojoba flour.

Jojoba flour was obtained from EMEC Agro Industries (Antwerp, Belgium).

Jojoba flour was first extracted with hexane to remove residual oil. 1 kg de-oiled flour was extracted with acetone for 12 hours by means of a Soxhlet apparatus. After evaporation of the solvent 40 g of a brown residue was obtained. The residue was dissolved in methanol and adsorbed onto 100 g of silica gel (0.2-0.5 mm). The methanol was removed under vacuum and the silica gel placed on top of a silica gel column (length 30 cm, diameter 6 cm) which contained a suspension of 1 kg silica gel (0.040-0.063 mm) in chloroform. The column was first eluated with 1 litre chloroform and the organic solvent thrown away, followed by elution with acetone. Fractions of 100 ml were collected and analyzed by means of TLC until simmondsine and analogue compounds were fully eluated. The first fractions contain mostly simmondsine-2'-ferulate, followed by mixtures of decreasing amounts of simmondsine and simmondsine analogues. Simmondsine-rich fractions were collected and filtered over active carbon, resulting in a light yellow solution. After evaporation of the acetone the crude extract (29 g) was further purified on another silica gel column (length 60 cm, diameter 6 cm) containing a suspension of 2 kg silica gel (0.040-0.063 mm) in chloroform. A solution of the non-purified simmondsine in methanol was first adsorbed onto silica gel (0.2-0.5 mm) and after evaporation of the solvent placed on top of the column. Elution was carried out with a mixture of methanol and chloroform (5/95, v/v). The eluates were guided through an UV cell to immediately determine the eluting compounds and corresponding fractions were separately collected. All peaks were examined for the presence of simmondsine and analogues by HPLC. The fractions containing only simmondsine were collected and the solvent

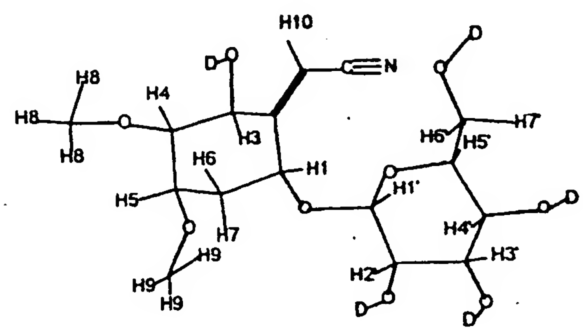
evaporated under vacuum. The residue (22 g) was crystallized from ethylacetate/methanol (1:3).

The yield of pure simmondsine amounted to 1.8% on the basis of the jojoba flour. The purity of simmondsine was checked by means of a TLC and an HPLC procedure. The TLC showed only one violet coloured spot and the HPLC analysis only one peak. The simmondsine was obtained as colourless crystals from ethylacetate.

The NMR results are shown in tables 19 and 20.

Table 19

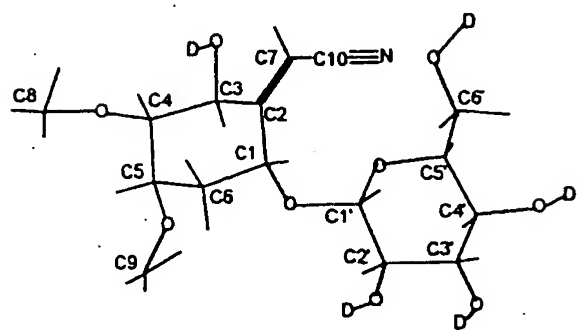
^1H NMR data for simmondsine (400 MHz in CD_3OD)



H1	4.87	t	J = 4 Hz
H3	4.72	dd	J3,4 = 9; J3,1 = 2
H4	3.12	dd	J4,3 = 9; J4,5 = 3
H5	3.90	q	J5,4 = 3
H6-H7	1.68-2.5	m	J6,1 = 3
H10	5.71	d	J7,3 = 2
3H8	3.43	s	
3H9	3.47	s	
H1'	4.38	d	J1'2' = 9
H2'	3.22	dd	J2'1' = 7,7; J2'3' = 9
H3'	3.35	dd	J3'2' = 9; J3'4' = 9
H4'	3.28	dd	J4'3' = 9; J4'5' = 9
H5'	3.22	ddd	
H6'	3.62	dd	J6'5' = 5; J6'7' = 11
H7'	3.82	dd	J7'6' = 11; J7'5' = 2

Table 20

^{13}C NMR data for simmondsine (100 MHz in CD_3OD)



C1	76.8	C1'	104.1
C2	166.4	C2'	74.6
C3	70.8	C3'	78.2
C4	86.4	C4'	71.5
C5	76.5	C5'	78.2
C6	32.1	C6'	62.8
C7	95.2		
C8	58.2		
C9	58.5		
C10	117.6		

CLAIMS

1. The use of simmondsine in a suitable form and dose for influencing the food intake by animals such as fish, birds and mammals.

2. Use according to claim 1, **characterized in that**, the simmondsine is substantially pure simmondsine and/or one or more derivatives and/or one or more analogues thereof.

3. Use according to claim 2, **characterized in that**, the analogue of simmondsine is simmondsine-2'-ferulate.

4. Use according to claim 2, **characterized in that**, the derivative of simmondsine is an aglucon of simmondsine.

5. Use according to claim 1, **characterized in that**, the simmondsine takes the form of optionally heated and/or de-oiled jojoba flour.

6. Use according to claim 5, **characterized in that**, the jojoba flour contains a simmondsine extract from the jojoba plant as additive.

7. Use according to claim 1, **characterized in that**, the animals are live-stock mother animals.

8. Use according to claim 7, **characterized in that**, the live-stock mother animals are chick-mother animals.

9. Use according to claim 8, **characterized in that**, simmondsine in the form of 1-10% jojoba flour is admixed in the food for influencing the food intake by chick-mother animals.

10. Use according to claim 8, **characterized in that**, an amount of simmondsine active substances whose activity corresponds to 0.075-0.50% pure simmondsine is admixed in the food for influencing the food intake by chick-mother animals.

11. Use according to claim 9 or 10, **characterized in that**, the administering of simmondsine takes place in the life week 3 to life week 23 of the chick-mother animals.

12. Use according to claim 1, **characterized in that**, the animals are household pets.

13. Use according to claim 12, **characterized in that**, pure simmondsine is used in a concentration from 0.05-1.0%.

14. Use according to claim 12, **characterized in that**, the possibly de-oiled and/or heated jojoba flour is used in a concentration of 0.5-20%.

15. Use according to claim 1, **characterized in that**, the animals are animals for slaughter.

16. Use according to claim 15, **characterized in that**, a quantity of simmondsines with a simmonsine activity of 0.1-0.25% pure simmondsine is used.

17. Use according to claim 15, **characterized in that**, a 2-5% possibly de-oiled and/or heated jojoba flour is used.

18. Method for isolating and purifying simmondsine and/or derivatives and/or analogues thereof, comprising the steps of:

a) extracting de-oiled jojoba flour in order to obtain an extract;

b) separating the extract into fractions by means of adsorption chromatography;

c) filtering the fractions containing the simmondsine and/or derivatives and/or analogues thereof to obtain a filtrate;

d) separating the filtrate into fractions again by means of adsorption chromatography; and

e) crystallizing the simmondsine and/or derivatives and/or analogues thereof.

19. Method as claimed in claim 18, **characterized in that**, the extract respectively the filtrate is concentrated prior to the chromatography step.

20. Method as claimed in claim 18 or 19, **characterized in that**, the extraction is performed with

acetone, the first chromatography takes place on a silica gel column with acetone as eluant, the filtration takes place over activated carbon and the second chromatography takes place on a silica gel column with chloroform/methanol (95:5) as eluant.

21. Method as claimed in claim 19 or 20, **characterized in that**, concentration takes place by means of dissolving the extract respectively the filtrate in methanol and subsequently evaporating the methanol under vacuum.

22. Use of simmondsine as a substance for suppressing the appetite in humans.

23. Use of simmondsine as a substance for stimulating weight loss in adolescents.

24. Method for controlling the growth of animals comprising the concurrent steps of:

a) reducing the food intake of at least one animal over a period of time; and

b) administering to said at least one animal an amount of simmondsine effective to increase the feeling of appetite satisfaction in said at least one animal,

wherein the resulting concurrent food intake reduction and simmondsine administration together effect the desired controlled animal growth.

25. Method according to claim 24 wherein said simmondsine is pure simmondsine.

26. Method according to claim 24 wherein said simmondsine is present in a quantity of jojoba extract.

27. Method according to claim 24 wherein said simmondsine is present in a quantity of de-oiled jojoba flour.

28. Method according to claim 24 wherein said period of time is between 1 day and 1 year.

29. Method according to claim 24 wherein said amount of simmondsine is also effective to decrease the T3 and T4 plasma levels of said at least one animal when said simmondsine is admixed in food for said animal at a concentration of 0.075-0.50% pure simmondsine.

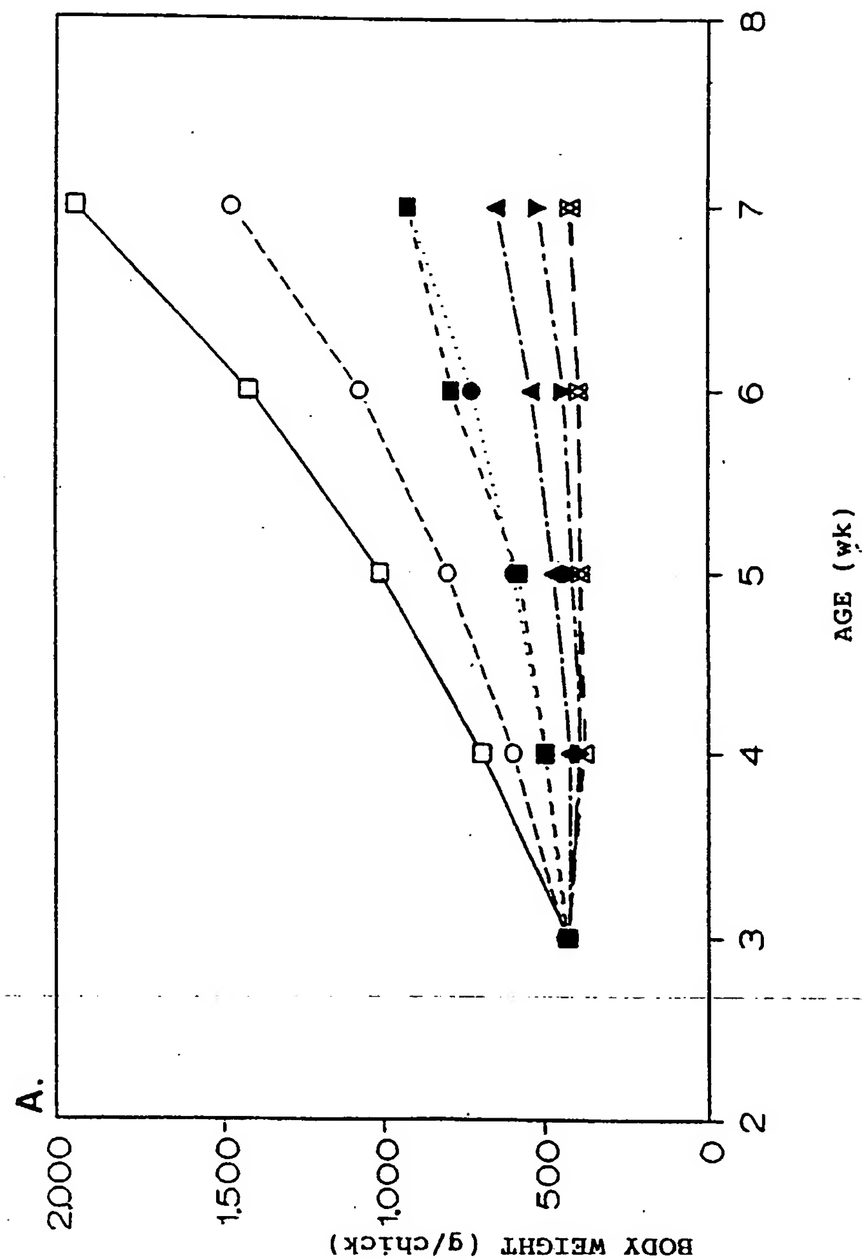


FIG. 1a

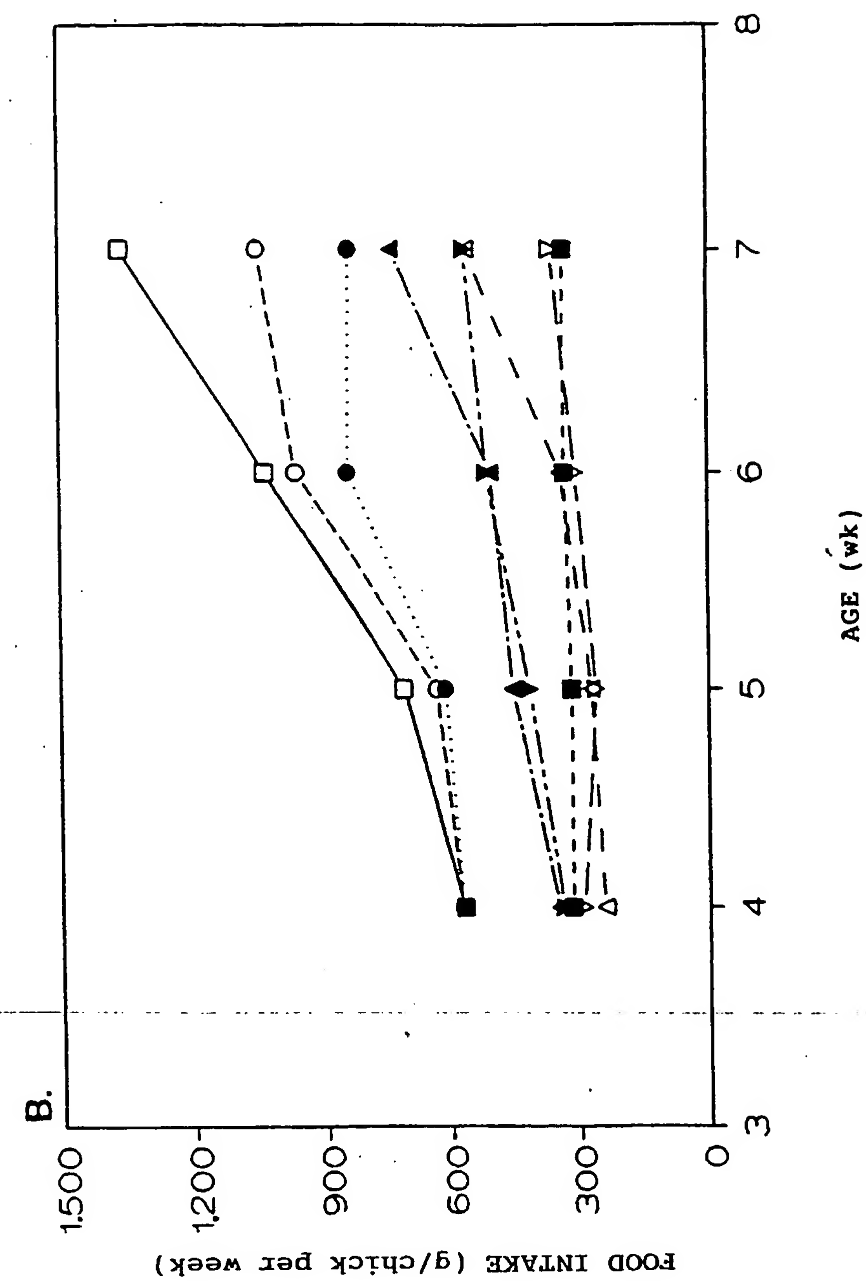

B.
FOOD INTAKE (g/chick per week)
1.500
1.200
900
600
300
0
3
4
5
6
7
8
AGE (wk)

FIG. 1b

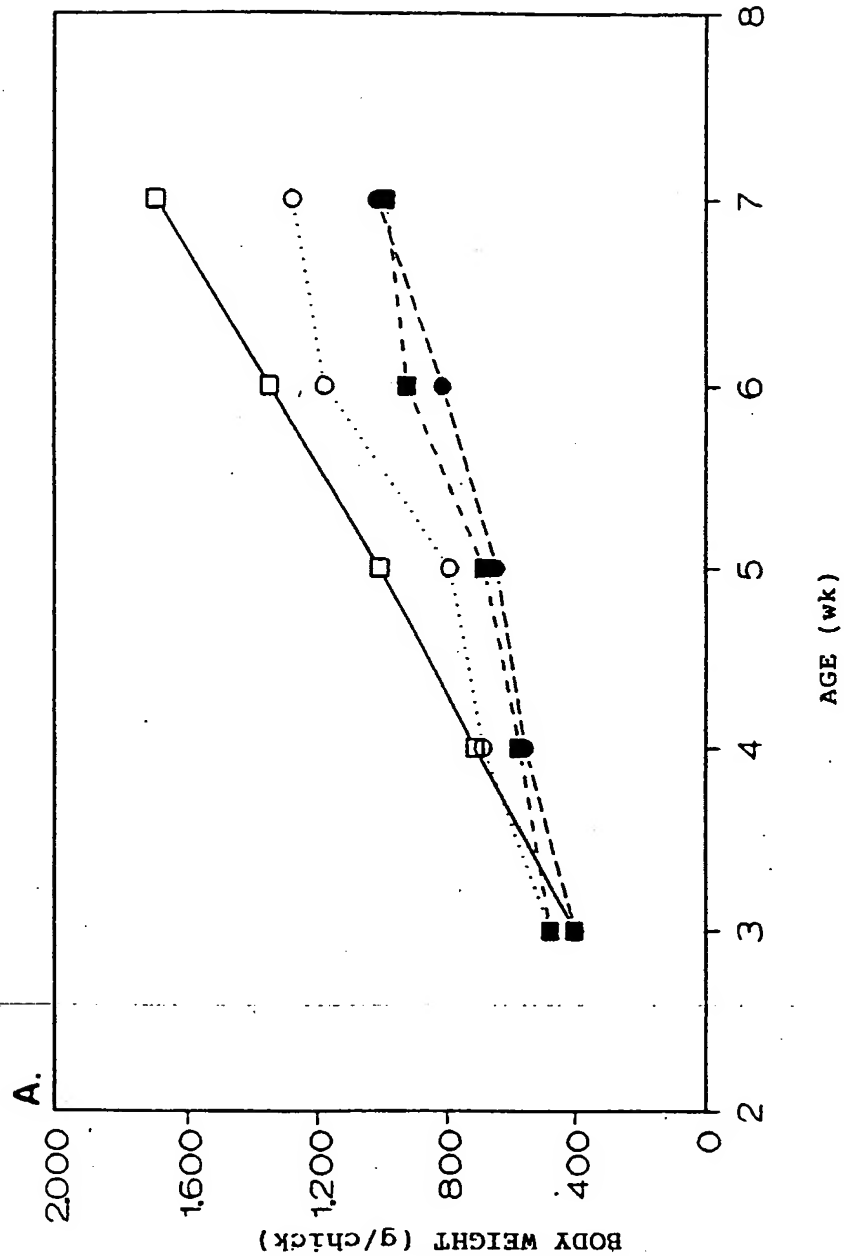


FIG. 2a

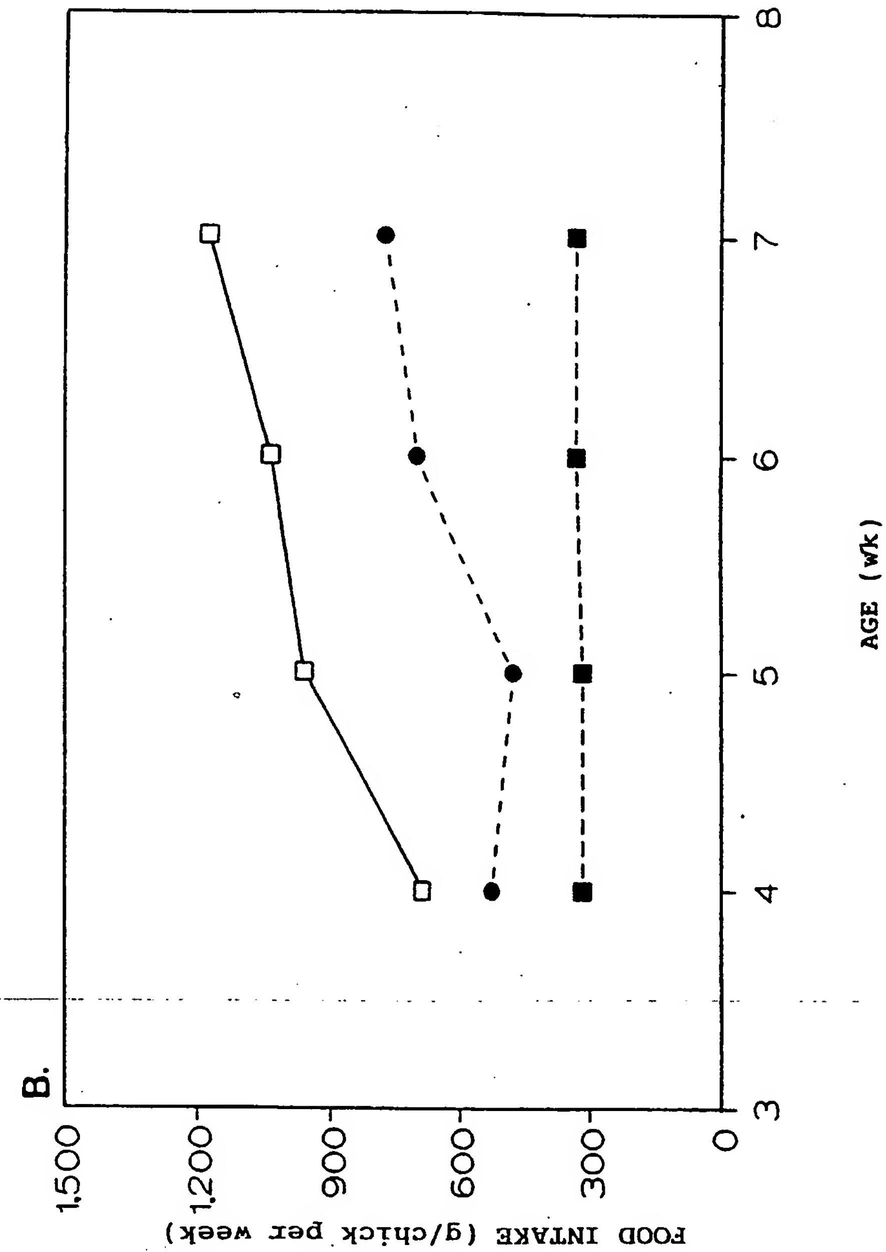

B.
FOOD INTAKE (g/chick per week)
1,500
1,200
900
600
300
0
3
4
5
6
7
8
AGE (wk)

FIG. 2b

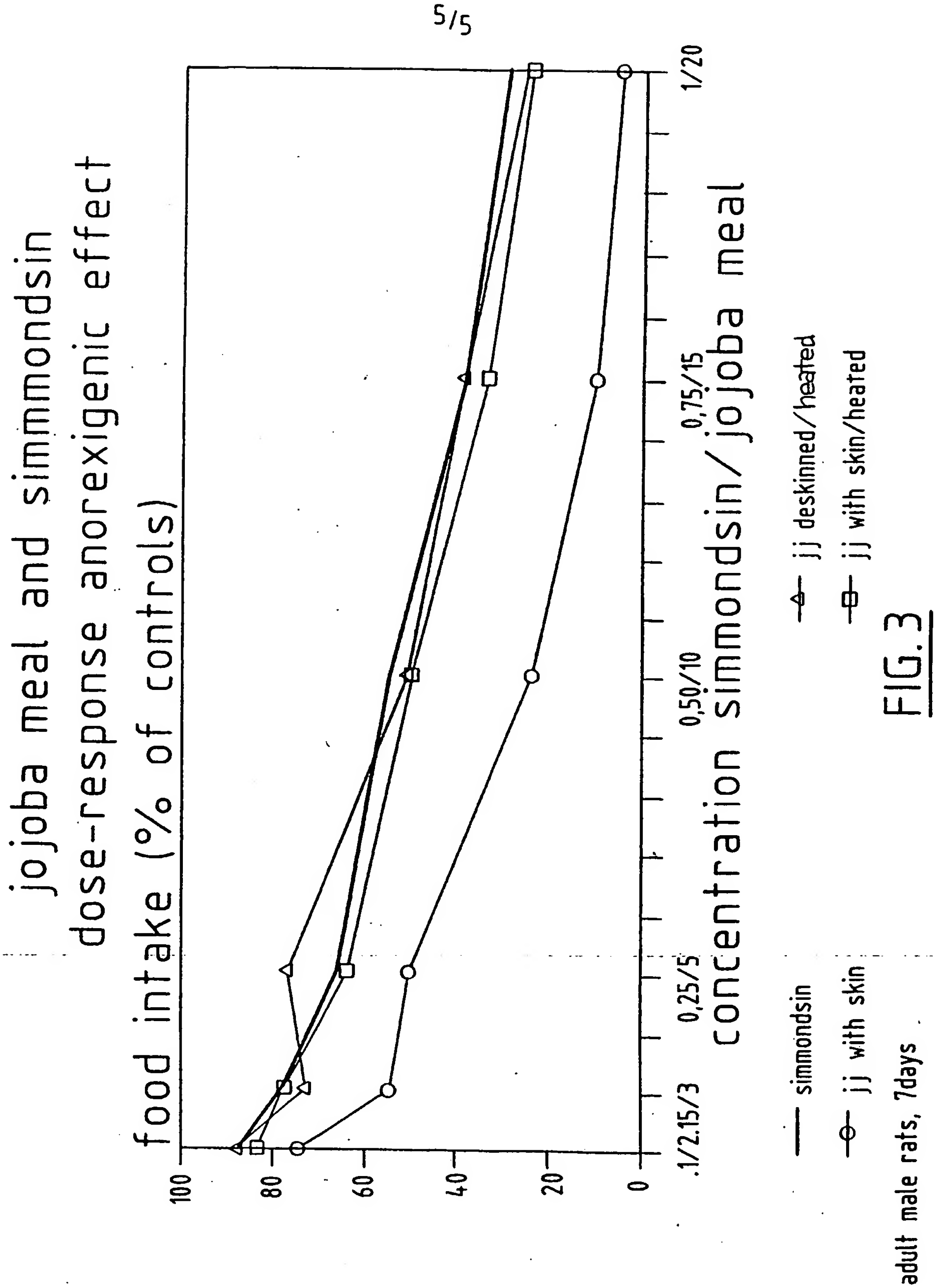

jojoba meal and simmmondsin
dose-response anorexigenic effect
food intake (% of controls)
100
80
60
40
20
0
1/2.15/3
0,25/5
0,50/10
0,75/15
1/20
concentration simmondsin/jojoba meal
simmondsin
jj with skin
jj deskinned/heated
jj with skin/heated
adult male rats, 7days

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 94/01434

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/70 C07H15/203 A23K1/16 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K C07H A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NL,A,8 901 639 (HUGO DANGREAU ET AL.) 2 January 1991 see page 1, line 1 - page 4, line 30 see examples I-V see claims 1-13 ---	1-4, 22-25
X	DE,A,35 31 380 (LA JOJOBA OIL) 12 March 1987 see column 2, line 31 - column 3, line 34 see column 3, line 55 - column 4, line 56 see claims 1,6-10 ---	1,2, 22-25

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
18 August 1994	26.08.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Dekeirel, M

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International application No.
PCT/EP 94/01434

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	POULTRY SCIENCE, vol.72, no.9, 1993, US pages 1714 - 1721 S. ARNOUTS ET AL. 'Jojoba meal (Simmondsia chinensis) in the diet of broiler breeder pullets: Physiological and endocrinological effects' see the whole document ---	1,5,7-9, 11,12, 14,15, 17,24, 26-29
P,X	JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol.41, no.10, 1993, WASHINGTON US pages 1605 - 1607 M. VAN BOVEN ET AL. 'Isolation, purification, and stereochemistry of simmondsin' see the whole document ---	18-21
A	POULTRY SCIENCE, vol.61, 1982, US pages 1692 - 1696 J.D.NGOU NGOUPAYOU ET AL. 'Jojoba meal in poultry diets' cited in the application see page 1962, column 2, paragraph 4 see page 1963, column 2, paragraph 3 - page 1964, column 2, paragraph 1 see page 1964, column 1; table 2 see page 1965; table 3 see page 1696, column 1, last paragraph ---	1-17, 24-29
A	JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol.40, no.10, 1992, WASHINGTON US pages 1839 - 1842 M.M. COKELAERE ET AL. 'Influence of pure simmondsin on the food intake in rats' cited in the application see the whole document ---	1-29
A	LIFE SCIENCES, vol.15, no.6, 1974, US pages 11115 - 11120 A.N. BOOTH ET AL. 'Isolation of a toxic factor from jojoba meal' see page 11117, last paragraph ---	18
A	CEREAL CHEMISTRY, vol.64, no.2, 1987, MINNEAPOLIS US pages 91 - 93 MEGANNE O. WISEMAN ET AL. 'Characterization of protein concentrates of jojoba (Simondsia chinensis) meal' see page 92, column 2, paragraph 4 -----	18

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/EP 94/01434

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
NL-A-8901639	02-01-91	NONE	
DE-A-3531380	12-03-87	NONE	